UNIVERSITY OF MARYLAND

Condensed NMR Hands-on Operation Manual

Supplement and suitable only for training by T.A. or Senior Peer

Full version is available at http://chem.umd.edu/nmr

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Caution and Reminder:

You are working very close to several strong super-conducting magnets in the NMR lab. Follow all safety procedures and policiesy as disclosed to you at the safety orientation.

To reserve spectrometer*: Go to http://www2.chem.umd.edu/nmr*

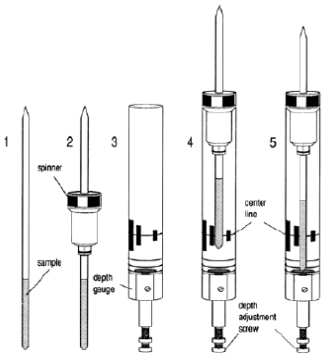
PLANNING ----- Your NMR measurements.
Contact staff for additional assistance. Appointment is highly recommended for project consultation.

Basic sample preparation:

- **NMR Tube**: 5mm OD, 7" long. Quality grade at least for 400MHz field strength.
- **Amount of compound**: (assuming M.W. < 1,000, soluble at room temperature, and pure)
    - Solid compounds: ~ 1-- 4 mg for H-1; ~ 10 – 40 mg for C-13.
    - Liquid compounds: ~ 0.1 – 0.2 Volume % for H-1, ~ 5 – 15 % for C-13.
- **Volume of Solvent**: ~ 0.5 to 0.6 ml. Deuterated solvent. Avoid mixed solvents.

Prior to NMR measurement:

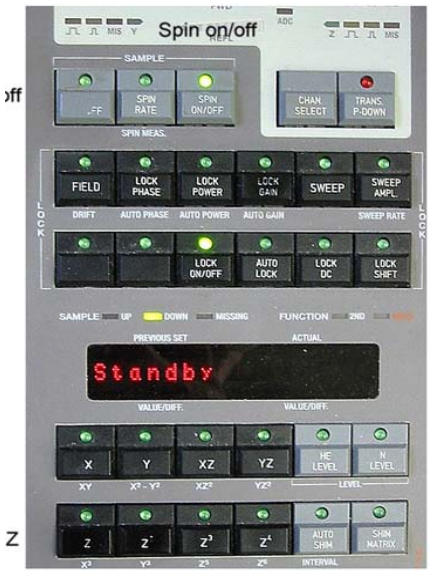
- **Insert NMR sample tube into a special holder :**
- Select a blue plastic sample holder (also known as **spinner**).
- Follow the recommended procedure as shown in the safety orientation.
- Adjust proper position of the sample:



Improper adjustment leads to no detectable signals or breaking the NMR tube inside the detector.

- **Insertion of NMR sample (with spinner) to the magnet:**

1. Press “LIFT” button on the keypad (see description below).
2. A standby sample, if any, will slowly rise up to the top of the magnet.
3. Replace the sample with yours. Before releasing it, ensure the tube & spinner are supported by the compressed air from the magnet entry port.
4. Press the “LIFT” button again to lower your sample into the magnet.



The Keypad: Located next to the console Computer.

The layout consists of a matrix of buttons.
By pressing a specific button, a certain function can be modified by turning the wheel-knob.

The LED flashes if a selected function fails.

The value of adjustment is shown on the center LED bar.

Three major groups: details will be described later in the instruction sessions.

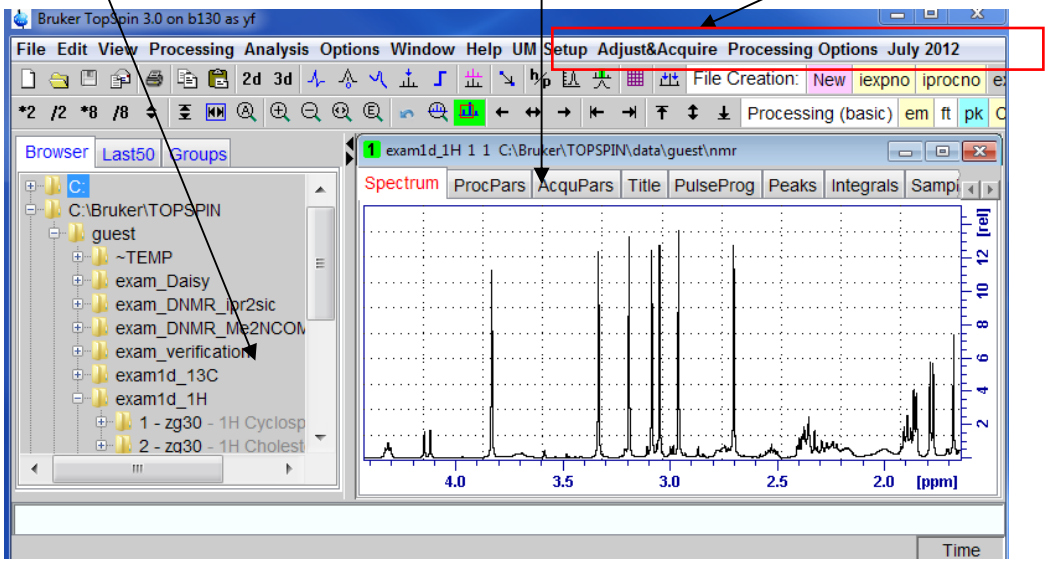
1. Mechanical control on NMR sample.
2. Field controls (lock, sweep).
3. Shim gradient adjustments (details see Appendix A).

TO start **TOPSPIN®,** double click on PC desktop,

NMR Data:

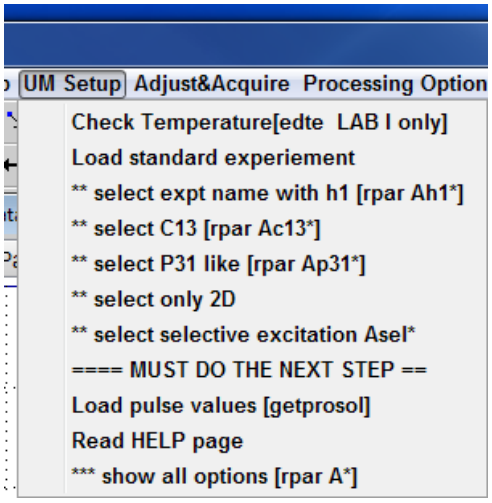
UM custom icons and pull down menu

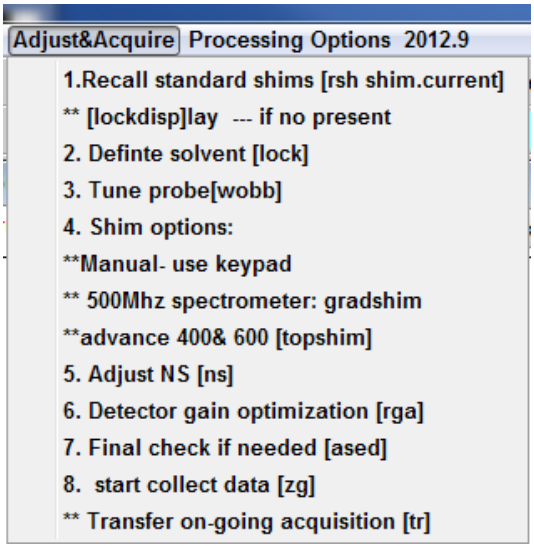
Parameters

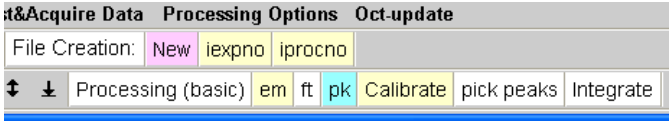


To execute a command: 3 options

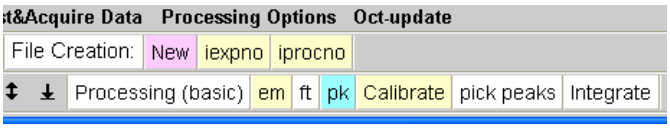
a) With the mouse: point; click; and/or drag. Icons.
b) Click the command line and type in the command via key board!
c) Use UM pull down menu or icons, examples as shown below


UM Setup Adjust&Acquire Processing Option
Check Temperature[edte LAB I only]
Load standard experiement
** select expt name with h1 [rpar Ah1*]
** select C13 [rpar Ac13*]
** select P31 like [rpar Ap31*]
** select only 2D
** select selective excitation Asel*
==== MUST DO THE NEXT STEP ==
Load pulse values [getprosol]
Read HELP page
*** show all options [rpar A*]


Adjust&Acquire Processing Options 2012.9
1.Recall standard shims [rsh shim.current]
** [lockdisp]lay --- if no present
2. Definte solvent [lock]
3. Tune probe[wobb]
4. Shim options:
**Manual- use keypad
** 500Mhz spectrometer: gradshim
**advance 400& 600 [topshim]
5. Adjust NS [ns]
6. Detector gain optimization [rga]
7. Final check if needed [ased]
8. start collect data [zg]
** Transfer on-going acquisition [tr]


t&Acquire Data Processing Options Oct-update
File Creation: New iexpno iprocno
Processing (basic) em ft pk Calibrate pick peaks Integrate

Create a DATA file: Click or type "NEW"

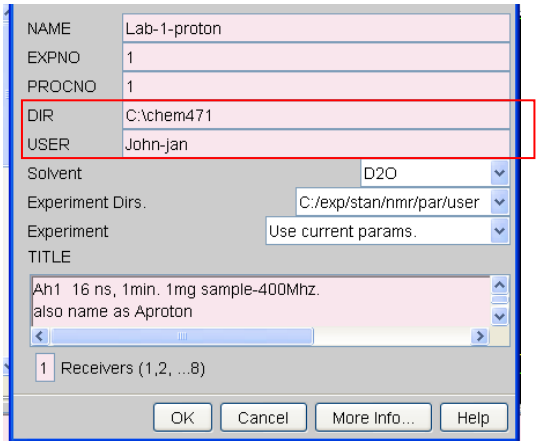

t&Acquire Data Processing Options Oct-update
File Creation: New iexpno iprocno
Processing (basic) em ft pk Calibrate pick peaks Integrate

a. **Name:** Define the name to identify your sample. e.g. sample813

b. **EXPNO:** "experiment number"…. Any integer [1 ….. 999].

c. **PROCNO:** 1

d. **Dir:** C:\reserach-group



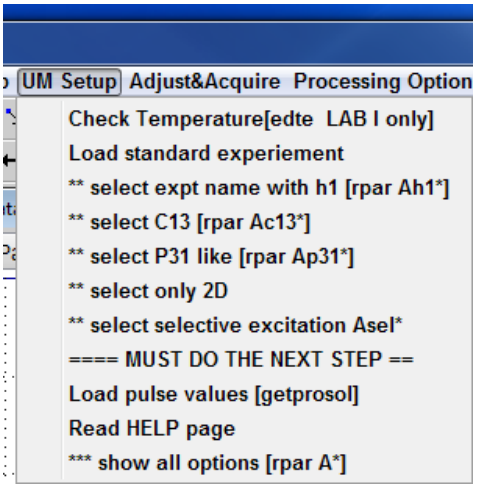
e. **USER:** Mandatory format
"Your-name-month".
where month is when the data sets are created.

f. **Solvent:** click and select the solvent in your sample.

g. **Mandatory to keep the next two entries as shown in the figure.**

i. **Experiment Dirs**: "C:/Bruker/TOPSPIN/exp/stan/nmr/par/user".
ii. **Experiment:** "Use current params".

Basic Setup procedure:


UM Setup Adjust&Acquire Processing Option
Check Temperature[edte LAB I only]
Load standard experiement
** select expt name with h1 [rpar Ah1*]
** select C13 [rpar Ac13*]
** select P31 like [rpar Ap31*]
** select only 2D
** select selective excitation Asel*
==== MUST DO THE NEXT STEP ==
Load pulse values [getprosol]
Read HELP page
*** show all options [rpar A*]

After you create a file and with your sample properly inserted into the magnet:

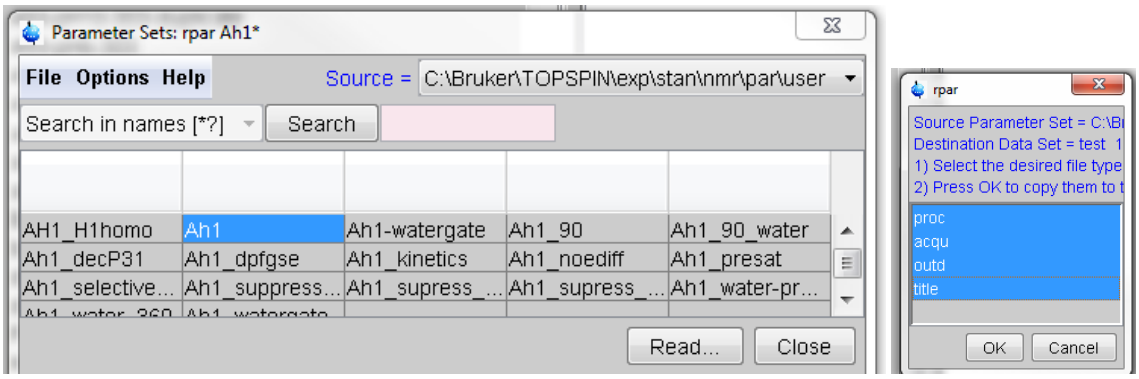
Click on UM Setup, to select and load standard experiment.

Format: " * " wild card represents any characters.

[rpar Ah1*] shows all applications with a title that contains any characters with "h1", proton measurements.

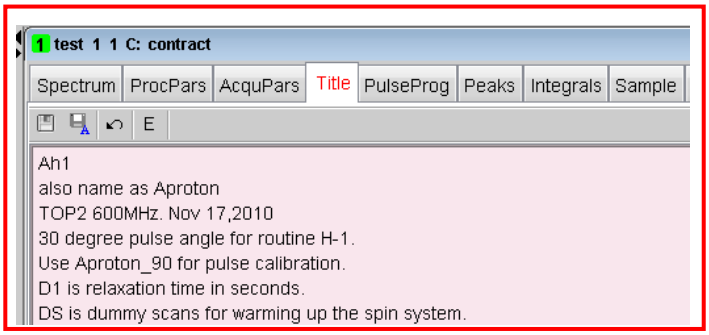
[rpar Ac13*] shows all applications with a title that contains "c13". e.g. c13cpd for C13 measurement with H-1 decoupled.

[**getprsol**] "Load pulse values" specific for the spectrometer being used. Neglecting to load the current values will not give proper NMR measurement.



Basic procedure for a select NMR measurement:

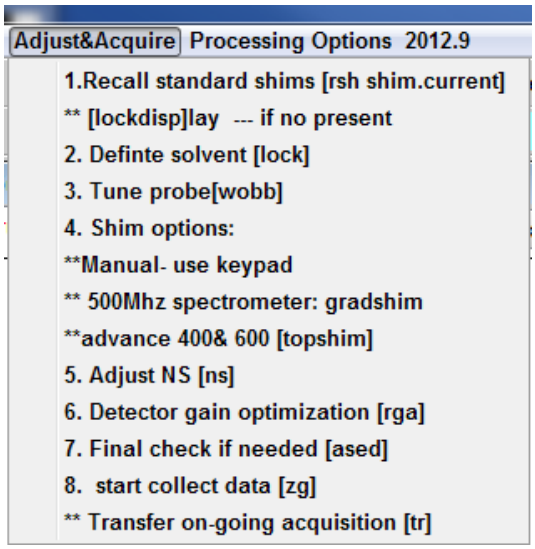
[**edti**] Read instruction for the selected measurement. Once you review them, one may replace the content with your comments relevant to your sample.



Or click the title page once you finish loading the parameter.

Optimization and data acquisition --- Adjust and Acquire menu:

Execute the steps in sequence from top to bottom.


Adjust&Acquire
Processing Options 2012.9
1.Recall standard shims [rsh shim.current]
** [lockdisp]lay --- if no present
2. Definte solvent [lock]
3. Tune probe[wobb]
4. Shim options:
**Manual- use keypad
** 500Mhz spectrometer: gradshim
**advance 400& 600 [topshim]
5. Adjust NS [ns]
6. Detector gain optimization [rga]
7. Final check if needed [ased]
8. start collect data [zg]
** Transfer on-going acquisition [tr]

Skip Step 3 for routine spectrometer.

Tune probe [wobb]: Not applicable for routine spectrometers!

Optimization and data acquisition
(Continued)

Step 4 --- Manual Shim (Essential for obtaining high resolution sharp NMR signals).

Remark: *The procedure is the* basic simplified steps at UM facility only, where calibration of shim settings are updated weekly.

```
4. Shim options:
**Manual- use keypad
** 500Mhz spectrometer: gradshim
**advance 400& 600 [topshim]
5. Adjust NS [ns]
6. Detector gain optimization [rga]
7. Final check if needed [ased]
8.  start collect data [zg]
** Transfer on-going acquisition [tr]
```

✓ *Ultimate criteria:*

Always check the line shape with a quick single scan to obtain a H-1 spectrum after any shimming!

- ✓ ***Spinning: OFF for short or routine samples***
- ✓ ***(< 0.5ml)***
- ✓ ***Adjust your speed in shimming any gradient shim such that the lock level can response to the change.***
- ✓ ***During the course of shim adjustment, if the lock level exceeds the display scale, reduce the lock gain to a level ~ 80 % of the display window.***

I. Take note of the lock level in the lock display as the initial reference level.

II. To test which direction you should change $Z^{2:}$
   a. Start by reducing the Z^2 shim value as indicated in the key pad slightly by ~ 20 to 50 units. Very often the lock level may decrease slightly, don't worry!
   b. Adjust with Z shim in such manner as to increase the lock level by a few segments. Value of the Z shim (as indicated in the key pad) could increase or decrease.
   c. If the lock level has increased higher than the initial reference level, cycle steps a to b to achieve the highest possible maximum.
   d. The final level may vary from one sample to the others.

III. If level cannot be improved after step b:

   - o Reverse the adjustment of $Z^{2.}$ Namely, increase the Z^2 shim value by 20 to 50 units, and use the Z shim to increase for the lock level.
   - o Cycle Z^2 and Z a few times to obtain the highest possible lock level. (compared with the initial reference level).

IV. For best resolution, also adjust the X, Y and XY shims to obtain the maximum lock level.

V. Finally, collect a quick one scan proton spectrum and review for sharp and symmetrical line shape.

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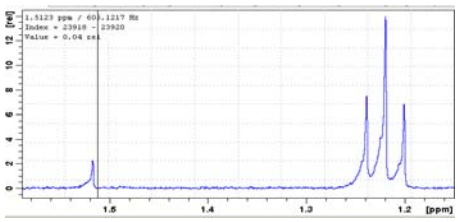
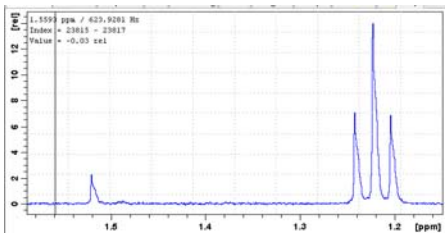
Common Examples of Poor Shimming:

Example ONE: All NMR signals have humps towards the large ppm (figure below) at their base.
Reason ----- Z^2 shim value is set **too large**.



Correction procedure: Use keypad, reduce the value of Z^2 shim ~ 25—50 units (lock level may drop slightly at this moment); re-adjust Z^1 shim to maximize the lock level; re-collect another H-1 spectrum. If the line shape improves and becomes more symmetrical, repeat the same adjustment sequence till the humps disappear.

Example TWO: Line humps towards the small ppm region.



Reason --- Z^2 shim value is set **too small**; not enough Z^2 current.
Reverse the correction procedure for example 1.

Follow the rest of steps (5 to 8) to collect data.

Step 6: *Receiver gain auto-adjust (for best digitizer scaling).*
Step 7: *Sample dependent parameters.*
Adjust as needed, otherwise use defaults.
D1, NS, and TDO.

D1 – relaxation delay for proper integration.

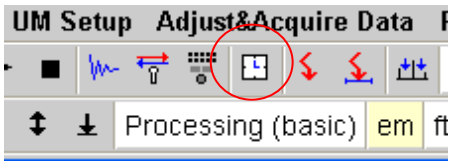
NS-- number of scans (concentration of your sample) Min is 8.

TD0 –Default set to 1 for H1. 5 or 10 for C13.
Final number of accumulated scans = NS * TD0

```
4. Shim options:
**Manual- use keypad
** 500Mhz spectrometer: gradshim
**advance 400& 600 [topshim]
5. Adjust NS [ns]
6. Detector gain optimization [rga]
7. Final check if needed [ased]
8.  start collect data [zg]
** Transfer on-going acquisition [tr]
```

**** [tr] optional step.**
******* to preview an on-going experiment (such as a C-13), before its completion.

To find out the acquisition time, type **[expt]** **`Or click time`**



- To halt the measurement ***`before it completes the scans, Type`*** **[halt] or click**
- ***DO NOT TYPE STOP!*** **[stop]** `will erase all the data collecte`

1D-NMR DATA PROCESSING:

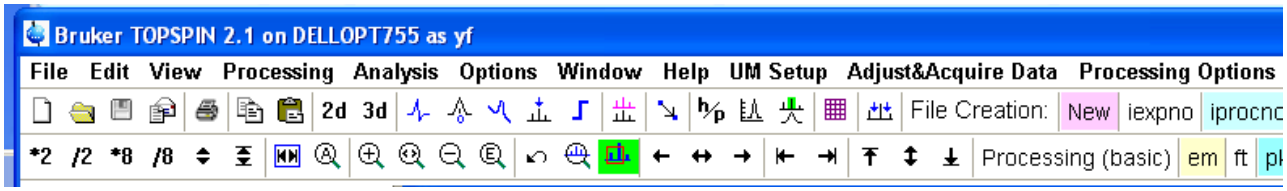
- *Ask your peer trainer or NMR T.A. for demo.*
- *Refer to Basic User manual Full version for details.*

Data transfer (from spectrometer to workstations):

Data file must be transferred with its corresponding filename.
Don't transfer an EXPNO subfolder alone that cannot be processed via TOPSPIN software.

Visual display adjustments & commands:

The content in the Spectrum window can be adjusted or processed by clicking/dragging the appropriate icon at the second &third rows: The UM short-cut (basic) commands are appended at the end of the 3rd row.



e.g. **Multiple displays:** to compare spectra**.**

Cut: Copy display to clipboard and paste to external word processing. Good for small expansion only. For comprehensive plot, details will be explained in the PLOT section.

For users who are not familiar with the software, click the UM Processing (basic) icons, in sequence, one by one, from left to right.

"em" Exponential multiplication; one of the most popular "apodization" in data processing.

Apply an exponential weighting function with a value of {LB}. A positive LB value will attenuate the FID data in an exponential manner. The noise of the spectrum will be reduced. Typical LB 0.3 (Hz) is used for H-1, and 2 to 4 Hz for C-13. *The larger the LB value, the greater amount of noise will be reduced, but the sharpness of signals will also be reduced [loss in resolution].*

" ft " Fourier transform FID.

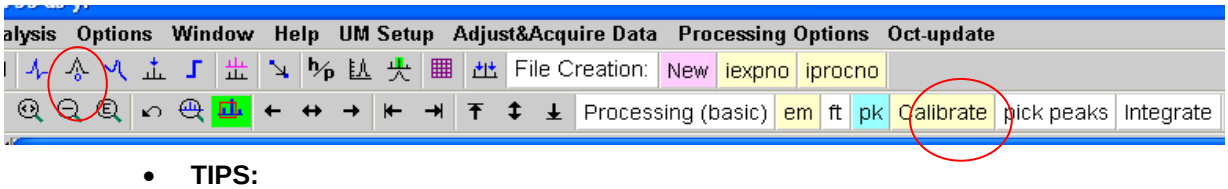
"apk" Automated reset phase

"pk" applies phase correction values { PHC0 and PHC1 } stored with the data set. Useful when identical phase adjustments are to be used.

When refined adjustment is required, click the icon to carry out phase correction manually

- *Ask your peer trainer or NMR T.A. for demo. You will be test this procedure on your check out!!!!!!!*

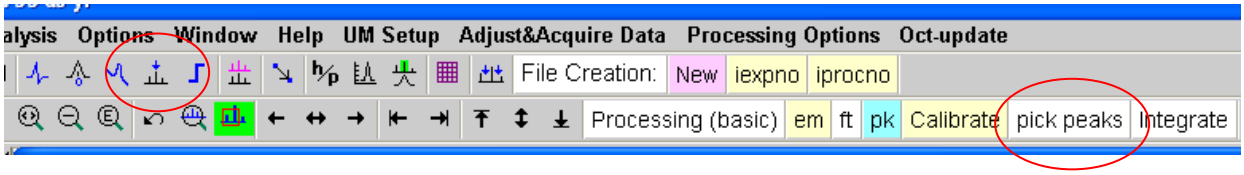
Chemical shift calibration, select and click either circled icons as shown in the figure --


alysis Options Window Help UM Setup Adjust&Acquire Data Processing Options Oct-update
File Creation: New iexpno iprocno
Processing (basic) em ft pk Calibrate pick peaks Integrate

- **TIPS:**

- Expand the spectrum first around the signal to be used for chemical shift calibration.
- IF your sample contains no standard reference signal (such as TMS), use the residual H-1 signal from the solvent. This method is acceptable but not a universal standard. Some solvent signals may shift with temperature or pH.

- When solvent doesn't give any residual H-1 or C13 signals**,** the **external reference** method is to be used.
  (Approximation method):
  - Prepare a reference sample that contains a suitable chemical with a known chemical shift value in the same solvent as your sample.
  - Collect a 1 D NMR spectrum at the same temperature as your sample.
  - Calibrate the shift and type " **sr** " to obtain a value known as the spectral reference .
  - Return to your original spectrum data file, import the value of sr obtained from the reference sample.


alysis Options Window Help UM Setup Adjust&Acquire Data Processing Options Oct-update
File Creation: New iexpno iprocno
Processing (basic) em ft pk Calibrate pick peaks Integrate

Peak Picking:

- **Option: Click the pick peaks icon or the** in software top menu row.
- Remark: Chemical shift calibration must be carried out before hand.
- Ask your peer trainer or NMR T.A. for demo.
- Refer to full User manual for description.

Option: Sensitivity of Peak picking factor "**pc** ". Pick peaking sensitivity can be modified with a parameter **pc**, The default value is 4.The smaller the value of PC, the larger the number of peaks will be indentified.

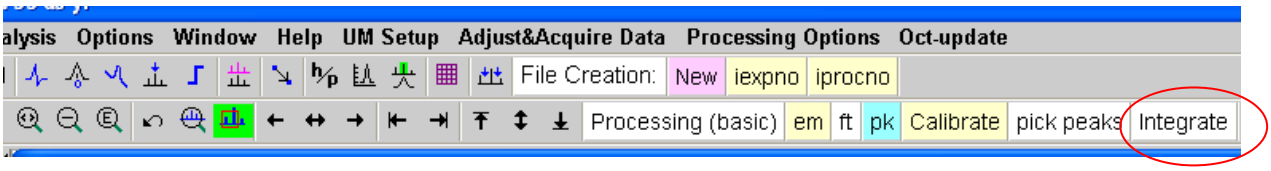
Integration:

Caution: For routine 1D H-1 NMR, proper integration will reflect the relative population of spins in a compound. *This is only true when the processing is done properly sufficient relaxation delay time (D1) when the data is collected.*

Procedure:

- Ask your peer trainer or NMR T.A. for demo.
- Refer to full User manual for description.

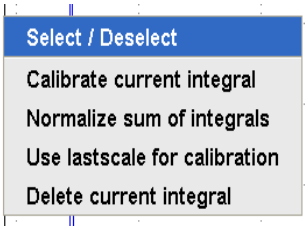
STEP 1: Auto integrate: Click the **integrate** icon or type [**abs] at command line.**

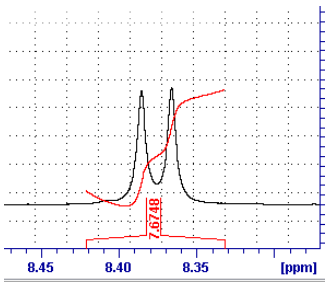

alysis Options Window Help UM Setup Adjust&Acquire Data Processing Options Oct-update
File Creation: New iexpno iprocno
Processing (basic) em ft pk Calibrate pick peaks Integrate

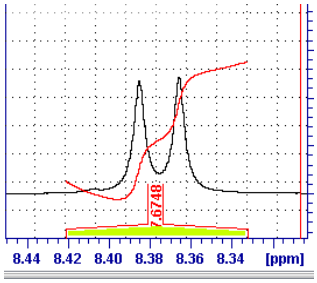
STEP 2: Modification and calibration (if needed) Click to review and/or modify the integrals:

Slope and bias on a particular Integral:

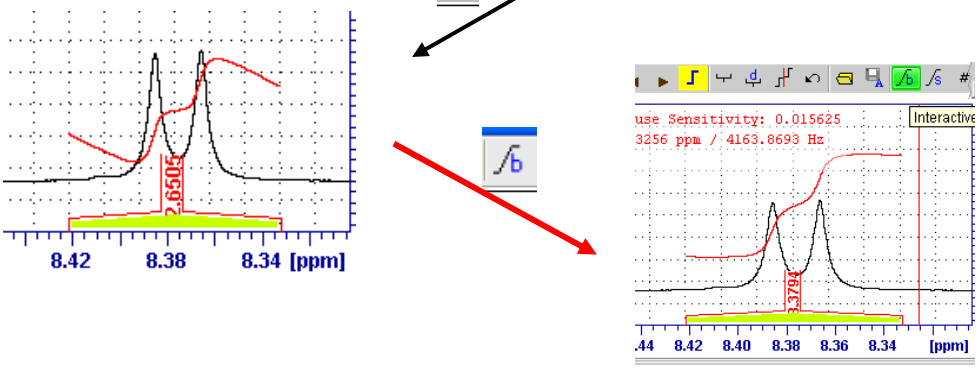
a) Select the region: Place the mouse on the integral of interest, right click and a pop-up menu appears. Click "Select/Deselect" once. The integral region turns yellowish-green.







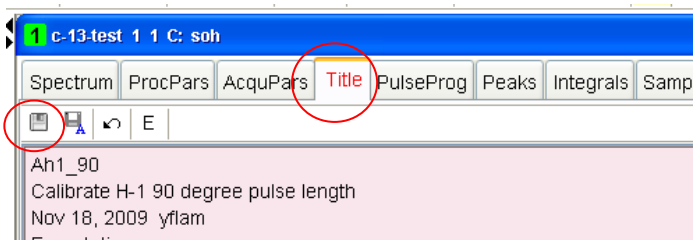
b) Adjust the slope then bias:



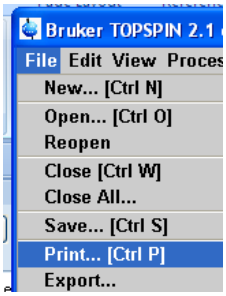
SAVE INTEGRATION: When all adjustments and modification are completed, calibrate the integral, and click "SAVE" to exit.

PLOT

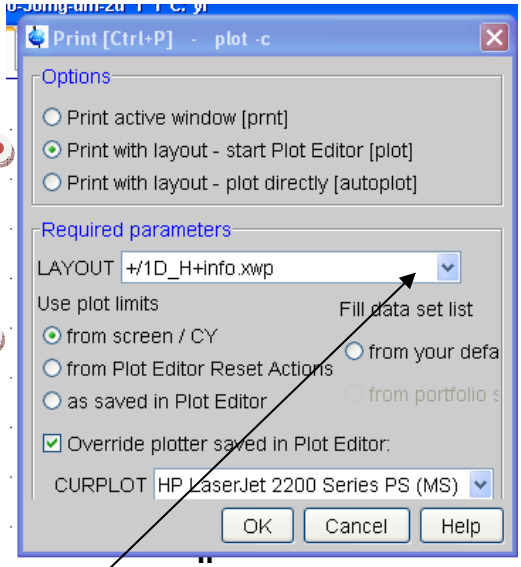
Before printing:
TAB the TITLE PAGE! Replace the original instruction with comment relevant to your sample measured. Click save when editing is completed.



Plot [print]: In top row of main window File >>>> click Print



A Pop-up option appears:



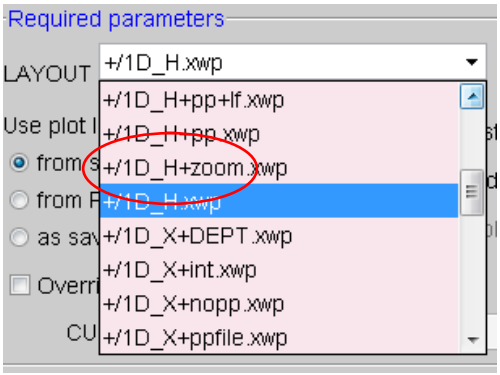
1. Select the three options as shown:
   - o If the LAYOUT is not desirable. Click the LAYOUT pull down menu and select a different LAYOUT.

What is a LAYOUT? It is a template that defines the properties of objects in a plot. The objects can be a spectrum, a list of parameters, a title, an image, or a combination of all. The object can be further modified in the plot editor.

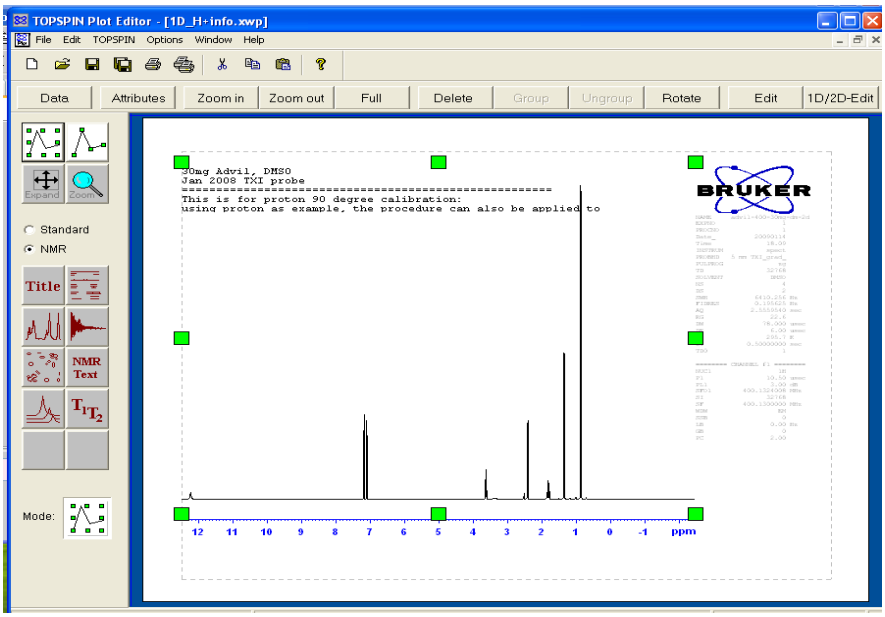
The most common choices are:

1D_H.xwp for proton; **1D_BB.xwp** for X-nuclei.

Stack_3.xwp for stack plots of 3 spectra.



2. Once OK is clicked. A new window “Plot Editor “appears,.

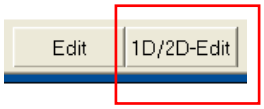

TOPSPIN Plot Editor - [1D_H+info.xwp]
File Edit TOPSPIN Options Window Help
Data Attributes Zoom in Zoom out Full Delete Group Ungroup Rotate Edit 1D/2D-Edit
Standard
NMR
Title
NMR Text
Mode:
30mg Advil, DMSO
Jan 2008 TXI probe
This is for proton 90 degree calibration:
using proton as example, the procedure can also be applied to
BRUKER
12 11 10 9 8 7 6 5 4 3 2 1 0 -1 ppm

Plot editor will automatically import the current spectrum as the main object defined in the layout.

- To modify the size and/or content of an object (such as the spectrum), place the mouse inside the object, left click to select.

- **Select the 1D/2D-Editor:**

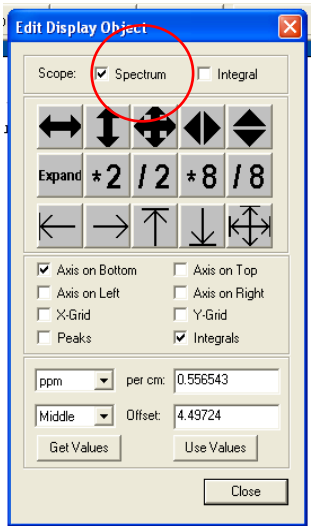
.



The Edit option (shown on the left) gives lots of options and is digital.

The **1D/2D-Edit option** is much easier to use!

Be sure to select either the Spectrum or the integral !
Then,



✓ When you finish modifying all the objects, click File and print within the Plot editor (**Don't use** the File- print in the main NMR software window).

✓ **CAUTION:** **When you exit (close) the Plot editor window, don't save the File when the software prompts. The file it's asking to save is the layout. It is not your spectrum.**

ABOUT THE Basic level NMR CHECK OUT (Open book): **Last update:** Oct, 2013.

Dead Line for your check out: _____

Name and signature of trainer: _____

- Basic expectation:

  a) Able to log on with your own User ID to activate the spectrometer.
  b) Able to compile all basic safety procedure (no loose metal objects; proper sample insertion) when working in the NMR lab.
  c) Able to collect a **properly shimmed** H1 spectrum within ten minutes.
  d) Able to FTP raw FID data to one of the workstations in the NMR Lab.

All data is to be processed and plot with TOPSPIN software and the workstations in NMR facility within TEN minutes.
Process the spectrum (properly phased and calibrate chemical shifts).

- *You will be tested use manual* *to do phase adjustment.*

- Basic understanding:
  You are expected to know the function of the essential terms: D1, NS, SW, em; ft; apk. (study Manual for details).

- Print two copies spectra with TOPSPIN software.

  a) The first plot must contain the following items—select layout: 1D_H1.xwp.

  - Acquisition & processing parameters listed.
  - Full spectral range.
  - No integration and peak. (Erase them if they appears in the plot screen).
  - Your name and USER ID included in the title.

  b) The second plot --- based on the first plot, delete the list of parameters, but add back the listing of shifts and integrations using the "1D/2D-Edit".

  - Make full use of the length of the chart paper for the plot.
  - The signals and the integrals are expended sufficiently large correspondingly, but the integrals are not overlapping the base line of the spectrum.
  - Pay particular attention on integrations over multiplex signals: proper slope; bias and range of integrals (study Manual for details).

Basic reference: www.chem.umd.edu/nmr